

PCT

WORLD INTEL



INTERNATIONAL APPLICATION PUBLISHED

WO 9603982A1

PCT

(51) International Patent Classification⁶:

A61K 31/15, 31/195, 31/22, 31/235,
31/34, 31/415, 31/66, 38/05, 38/06, C07K
5/02, 5/062, 5/083, C07C 243/26, 243/36,
243/38, C07D 231/04, 231/08, 231/20,
307/60

A1

(11) International Publication Number:

WO 96/03982

(43) International Publication Date:

15 February 1996 (15.02.96)

(21) International Application Number: PCT/US95/09668

(22) International Filing Date: 31 July 1995 (31.07.95)

(30) Priority Data:

08/284,861

2 August 1994 (02.08.94)

US

(71) Applicant: SANOFI WINTHROP, INC. [US/US]; 90 Park Avenue, New York, NY 10016 (US).

(72) Inventors: DOLLE, Roland, E.; 550 Prince Frederick Street, King of Prussia, PA 19406 (US). GRAYBILL, Todd, L.; 1380 Miller Road, Pottstown, PA 19464 (US).

(74) Agent: DAVIS, William, J.; Sanofi Winthrop, Inc., 9 Great Valley Parkway, Malvern, PA 19355 (US).

(81) Designated States: AU, CA, CN, FI, HU, JP, MX, NO, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

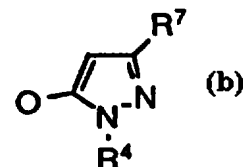
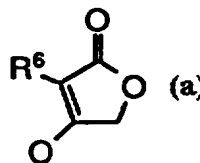
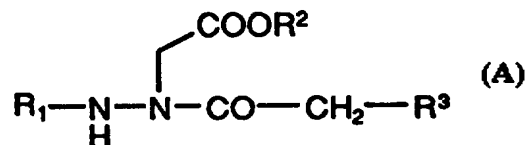
Published

With international search report.

(54) Title: AZAASPARTIC ACID ANALOGS AS INTERLEUKIN-1 BETA CONVERTING ENZYME INHIBITORS

(57) Abstract

Disclosed are compounds, compositions and methods for inhibiting interleukin-1 β protease activity. The compounds, α -substituted acetamides are of formula (A), wherein: R^2 = H or alkyl; R^3 = halo, $O(CO)_0-1$ aryl, $OPOR^4R^5$; (a), (b) where R^4 and R^5 = aryl; R^6 = H, aryl or aralkyl; R^7 = independently selected from R^6 , CF_3 and CF_2CF_3 ; R^1 = R^6 -CO, heteroaryl-CO, heteroaralkyl-CO and amino acid.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

**AZAASPARTIC ACID ANALOGS AS
INTERLEUKIN-1 *Beta* CONVERTING ENZYME INHIBITORS**

BACKGROUND OF THE INVENTION

5

Field of the Invention

This invention relates to a series of azaaspartic acid analogs which exhibit *in vitro* and *in vivo* inhibition of interleukin-1 β converting enzyme, to compositions containing the novel aspartic acid analogs and to methods for therapeutic utility. More particularly, the interleukin-1 β converting enzyme inhibitors described in this invention comprise novel azaaspartic acid α -substituted acetamides which possess particular utility in the treatment of inflammatory and immune-based diseases of lung, central nervous system, and connective tissues.

15

Reported Developments

Interleukin 1 β (IL-1 β) protease (also known as interleukin-1 β converting enzyme or ICE) is the enzyme responsible for processing of the biologically inactive 31 kD precursor IL-1 β to the biologically active 17 kD form (Kostura, M.J.; Tocci, M.J.; Limjoco, G.; Chin, J.; Cameron, P.; Hillman, A.G.; Chartrain, N.A.; Schmidt, J.A., Proc. Nat. Acad. Sci. (1989), **86**, 5227-5231 and Black, R.A.; Kronheim, S.R.; Sleath, P.R., FEBS Let. (1989), **247**, 386-391). In addition to acting as one of the body's early responses to injury and infection, IL-1 β has also been proposed to act as a mediator of a wide variety of diseases, including rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, sepsis, acute and chronic myelogenous leukemia and osteoporosis (Dinarello, C.A.; Wolff, S.M., New Engl. J. Med. (1993), **328**, 106). A naturally occurring IL-1 β receptor antagonist has been used to demonstrate the intermediacy of IL-1 β in a number of human diseases and animal models (Hannum, C.H.; Wilcox, C.J.; Arend, W.P.; Joslin, G.G.; Dripps, D.J.; Heimdal, P.L.; Armes, L.G.; Sommer, A.; Eisenberg, S.P.; Thompson, R.C., Nature, (1990), **343**, 336-340; Eisenberg, S.P.; Evans, R.J.; Arend, W.P.; Verderber, E.; Brewer, M.T.; Hannum, C.H.; Thompson, R.C., Nature (1990), **343**, 341-346; Ohlsson, K.; Bjork, P.; Bergenfeldt, M.; Hageman, R.; Thompson, R.C., Nature, (1990), **348**, 550-552; Wakabayashi, G., FASEB, (1991), **338-343**; Pacifici, R.; et al. Proc. Natl. Acad. Sci. (1989), **86**, 2398-2402 and Yamamoto, I.; et al. Cancer Res (1989), **49**, 4242-4246). The specific role of IL-1 β in inflammation and immunomodulation is supported by the recent observation that the

cowpox virus employs an inhibitor of ICE to suppress the inflammatory response of its host (Ray, C.A. et al, Cell, (1992), 69, 597-604).

5 The importance of these observations is well recognized by those skilled in the art and several workers have proposed and demonstrated *in vivo* the utility of ICE inhibitors in modifying certain IL-1 β mediated disease states. Some have suggested the development and therapeutic use of a small molecule inhibitor of mature IL-1 β formation (See, e.g., Miller, D.K. et al. "The IL-1 β Converting Enzyme as a Therapeutic Target" in Immunosuppressive and Antiinflammatory Drugs; Annals of the New York Academy of Sciences; Vol. 696, pp133-148, 1993). The following review of the current
10 state of the art in ICE research further supports such utility of ICE inhibitors:

1) WO 9309135, published 11 May 1993, teaches that peptide-based aspartic acid arylacyloxy-and aryoxymethyl ketones are potent inhibitors of ICE *in vitro*. These
15 compounds also specifically inhibited ICE in the whole cell (*in vivo*) by their ability to inhibit the formation of mature IL-1 β in whole cells. These ICE inhibitors also demonstrated utility in reducing fever and inflammation/swelling in rats.

2) Patients with Lyme disease sometimes develop Lyme arthritis. B. burgdorferi, the causative agent of Lyme disease, is a potent inducer of IL-1 synthesis by
20 mononuclear cells. Miller et al. (Miller, L.C.; Lynch, E.A. Isa, S.; Logan, J.W.; Dinarello, C.A.; and Steere, A.C., "Balance of synovial fluid IL-1 β and IL-1 Receptor Antagonist and Recovery from Lyme arthritis", Lancet (1993) 341; 146-148) showed that in patients who recovered quickly from Lyme Arthritis, the balance in synovial fluid
25 of IL-1 β and IL-1ra was in favor of IL-1ra. When the balance was shifted in favor of IL-1 β , it took significantly longer for the disease to resolve. The conclusion was that the excess IL-1ra blocked the effects of the IL-1 β in the patients studied.

3) IL-1 is present in affected tissues in ulcerative colitis in humans. In animal
30 models of the disease, IL-1 β levels correlate with disease severity. In the model, administration of IL-1ra reduced tissue necrosis and the number of inflammatory cells in the colon. See, Cominelli, F.; Nast, C.C.; Clark, B.D.; Schindler, R., Llerena, R.; Eysselein, V.E.; Thompson, R.C.; and Dinarello, C.A.; "Interleukin-1 Gene Expression, Synthesis, and Effect of Specific IL-1 Receptor Blockade in Rabbit Immune Complex
35 Colitis" J. Clin. Investigations (1990) Vol. 86, pp, 972-980.

- 4) The IL-1 receptor antagonist, Antril (Synergen), possess significant antiinflammatory activity in patients with active rheumatoid arthritis. In a multicenter Phase II dose ranging study, 175 patients received subcutaneous doses of Antril at 20mg, 70mg and 200mg seven times, three times or once per week. The antagonist
5 was found to be most effective when taken daily. After three weeks of daily treatment, patients showed a decrease in joint swelling and less disease activity (Scrip, NO 1873, 1993).
- 5) IL-1ra supresses joint swelling in the PG-APS model of arthritis in rats.
10 See Schwab, J.H.; Anderle, S.K.; Brown, R.R.; Dalldorf, F.G. and Thompson, R.C., "Pro- and Anti-Inflammatory Roles of Interleukin-1 in Recurrence of Bacterial Cell Wall-Induced Arthritis in Rats". Infect. Immun. (1991) 59; 4436-4442.
- 6) IL-1ra shows efficacy in a small open-label human Rheumatoid Arthritis trial.
15 See, Lebsack, M.E.; Paul, C.C.; Bloedow, C.C.; Burch, F.X.; Sack, M.A.; Chase, W., and Catalano, M.A. "Subcutaneous IL-1 Receptor Antagonist in Patients with Rheumatoid Arthritis", Arth. Rheum. (1991) 34; 545.
- 7) Soluble IL-1 receptor significantly reduces clinically the cutaneous late-phase
20 allergic reaction. This was demonstrated in a prospective, randomized, double-blind, placebo-controlled study on 15 allergic subjects. See, Mullarkey, M.F. et al. "Human Cutaneous Allergic Late-Phase Response is Inhibited by Soluble IL-1 Receptor", J. of Immunology, (1994) 152; 2033-2041.
- 8) IL-1 appears to be an autocrine growth factor for the proliferation of chronic
25 myelogenous leukemia cells. Both IL-1ra and sIL-1R inhibit colony growth in cells removed from leukemia patients. See, Estrov, Z.; Kurzrock, R.; Wetzler, M.; Kantarjian, H.; Blake, M.; Harris, D.; Gutterman, J.U.; and Talpaz, M., "Supression of Chronic Myelogenous Leukemia Colony Growth by Interleukin-1 (IL-1) Receptor Antagonist and
30 Soluble IL-1 Receptors: a Novel Application for Inhibitors of IL-1 Activity". Blood (1991) 78; 1476-1484.
- 9) As in 6) above, but for acute myelogenous leukemia rather than chronic myelogenous leukemia. See, Estrov, Z.; Kurzrock, R.; Estey, E.; Wetzler, M.; Ferrajoli, A.; Harris, D.; Blake, M.; Guttermann, J.U.; and Talpaz, M. "Inhibition of Acute Myelogenous Leukemia Blast Proliferation by Interleukin-1 (IL-1) Receptor Antagonist and Soluble IL-1 Receptors". (1992) Blood 79; 1938-1945.

An effective therapy has yet to be fully developed commercially for the treatment of IL-1 β mediated inflammatory diseases. Consequently, there is a need for therapeutic agents effective in the treatment and prevention of these diseases.

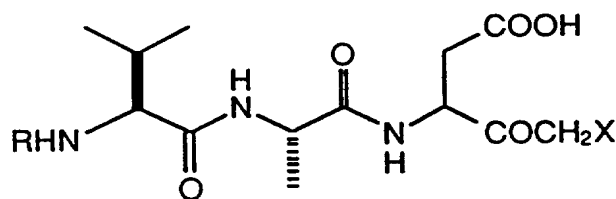
5

Summary of the Invention

It is known that aspartic acid is the P1 specificity determinant for ICE. This is exemplified by the potent irreversible inhibitor **i** (Figure 1) which contains a P1 aspartic acid residue. In this invention we describe inhibitors where the traditional aspartic acid residue is substituted with an azaaspartic acid residue as indicated by **ii** (Figure 1). In these novel agents, the α -carbon atom which bears the aspartic acid side chain has been replaced by a nitrogen atom.

15

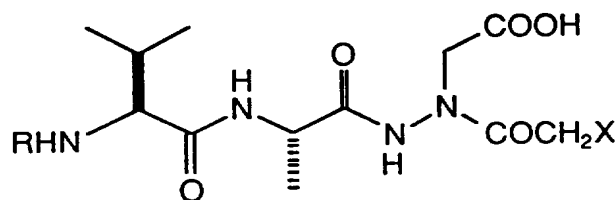
FIGURE 1



(i)

20

Aspartic acid-based ICE inhibitor
(Dolle, R.E. et al., J. Med. Chem. 37, 563 (1994))

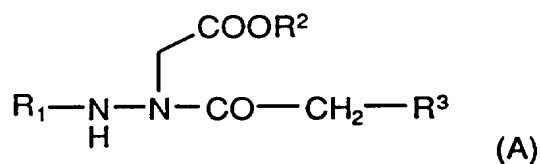


(ii)

25

Azaaspartic acid-based ICE inhibitor
(Present Invention)

According to the present invention, there is provided a compound of the formula (A) or a pharmaceutically acceptable salt thereof:



wherein:

$\text{R}_2 = \text{H}$ or alkyl;



where

R^4 and $\text{R}^5 = \text{aryl};$

$\text{R}^6 = \text{H}, \text{aryl}$ or aralkyl;

$\text{R}^7 =$ independently selected from R^6 , CF_3 and $\text{CF}_2\text{CF}_3;$

$\text{R}^1 = \text{R}^6\text{-CO}, \text{heteroaryl-CO}, \text{heteroaralkyl-CO}$ and amino acid.

"Alkyl" is defined as a saturated aliphatic hydrocarbon which may be either straight- or branched-chain. Preferred groups have no more than about 12 carbon atoms and may be methyl, ethyl and structural isomers of propyl, butyl, pentyl, hexyl, octyl, nonyl, decyl, undecyl, dodecyl.

"Aryl" is defined as a phenyl or naphthyl ring which may be substituted or unsubstituted wherein one or more of the hydrogen atoms has been replaced by the same or different substituents including halo, alkyl, aryl, nitro, cyano, amino, hydroxyl, alkoxy or haloalkyl.

"Aralkyl" means an alkyl radical substituted with an aryl ring. For example, benzyl, 4-chlorobenzyl.

"Heteroaryl" means pyridyl, thienyl or furanyl and structural isomers thereof.

"Heteroaralkyl" means an alkyl radical substituted by a heteroaryl ring. For example, 2-thienyl ethyl.

"Halo" means iodo, bromo, chloro, and fluoro.

5

"Amino acid" is defined as a commercially available natural or unnatural amino acid or dipeptide where the α -amino group in the amino acid or dipeptide has been protected with an N-protecting group. Such groups include, amides, sulfonamides, carbamates, and ureas as described in The Peptides, E. Gross and J. Meienhofer, Eds., Vol. 2, (1981) Academic Press, NY. N-Benzylloxycarbonyl-L-valine-L-alanine is an example.

10

The present invention also concerns the pharmaceutical composition and method of treatment of IL-1 β protease mediated disease states or disorders in a mammal in need of such treatment comprising the administration of IL-1 β protease inhibitors of formula (A) as the active agent. These disease states and disorders include: infectious diseases, such as meningitis and salpingitis; septic shock, respiratory diseases; inflammatory conditions, such as arthritis, cholangitis, colitis, encephalitis, endocervicitis, hepatitis, pancreatitis and reperfusion injury, immune-based diseases, such as hypersensitivity; auto-immune diseases, such as multiple sclerosis; bone diseases; and certain tumors and leukemias.

15

20

The present invention has particular utility in the modulation of processing of IL-1 β for the treatment of rheumatoid arthritis. Levels of IL-1 β are known to be elevated in the synovial fluid of patients with the disease. Additionally, IL-1 β stimulates the synthesis of enzymes believed to be involved in inflammation, such as collagenase and PLA2, and produces joint destruction which is very similar to rheumatoid arthritis following intra-articular injection in animals.

25

In the practice of this invention an effective amount of a compound of the invention or a pharmaceutical composition thereof is administered to the subject in need of, or desiring, such treatment. These compounds or compositions may be administered by any of a variety of routes depending upon the specific end use, including orally, parenterally (including subcutaneous, intraarticular, intramuscular and intravenous administration), rectally, buccally (including sublingually), transdermally or intranasally. The most suitable route in any given case will depend upon the use, the particular active ingredient, and the subject involved. The compound or composition

30

35

may also be administered by means of controlled-release, depot implant or injectable formulations as described more fully herein.

5 In general, for the uses as described in the instant invention, it is expedient to administer the active ingredient in amounts between about 0.1 and 100 mg/kg body weight, most preferably from about 0.1 to 30 mg/kg body weight for human therapy, the active ingredient will be administered preferably in the range of from about 0.1 to about 20-50 mg/kg/day. This administration may be accomplished by a single administration, by distribution over several applications or by slow release in order to achieve the most effective results. When administered as a single dose, administration will most preferably be in the range of from about 0.1 to mg/kg to about 10 mg/kg.

15 The exact dose and regimen for administration of these compounds and compositions will necessarily be dependent upon the needs of the individual subject being treated, the type of treatment, and the degree of affliction or need. In general, parenteral administration requires lower dosage than other methods of administration which are more dependent upon absorption.

20 A further aspect of the present invention relates to pharmaceutical compositions comprising as an active ingredient a compound of the present invention in admixture with a pharmaceutically acceptable, non-toxic carrier. As mentioned above, such compositions may be prepared for use for parenteral (subcutaneous, intraarticular, intramuscular or intravenous) administration, particularly in the form of liquid solutions or suspensions; for oral or buccal administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, nasal drops or aerosols.

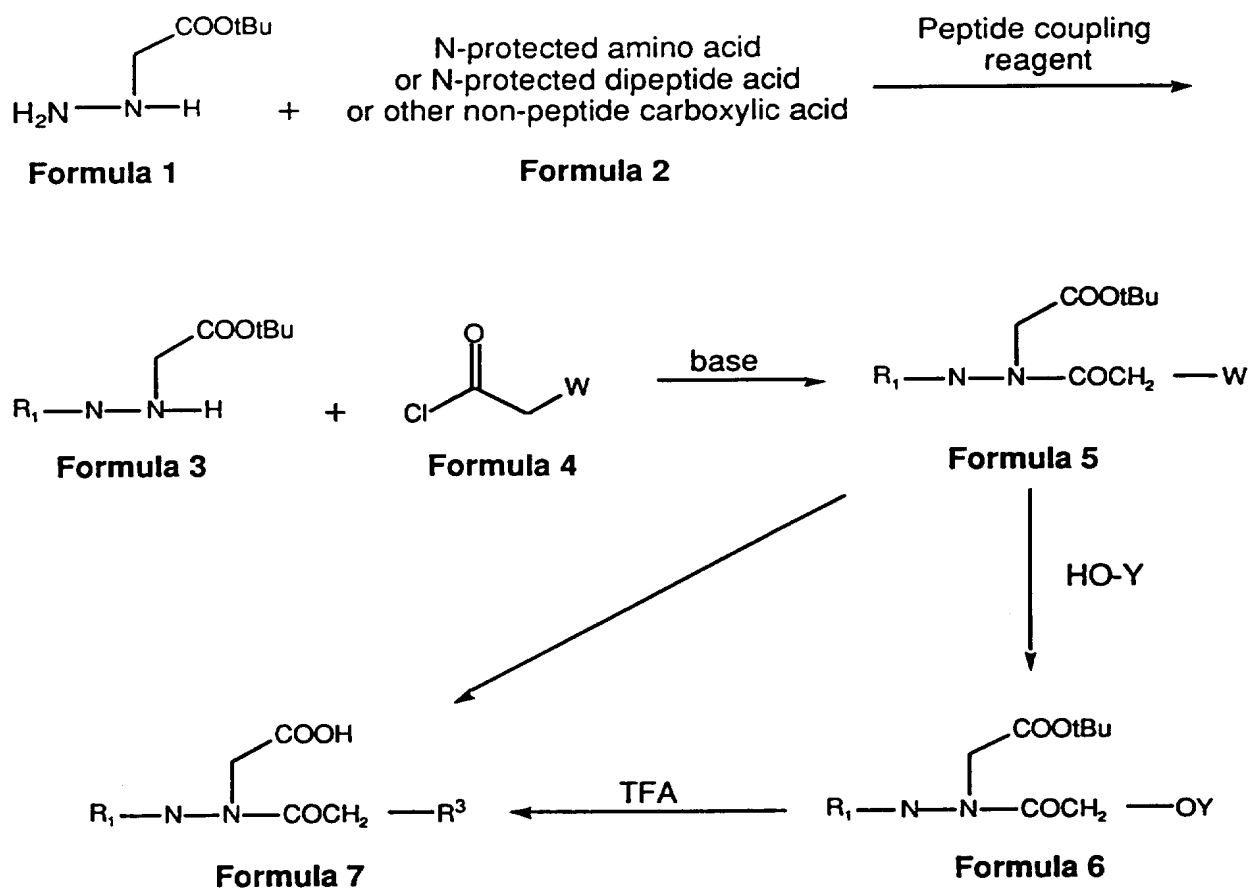
30 When administered orally (or rectally) the compounds will usually be formulated into a unit dosage form such as a tablet, capsule, suppository or cachet. Such formulations typically include a solid, semi-solid or liquid carrier or diluent. Exemplary diluents and vehicles are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, mineral oil, cocoa butter, oil of theobroma, alginate, tragacanth, gelatin, syrup, methylcellulose, polyoxyethylene sorbitar monolaurate, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, and magnesium stearate.

35

DETAILED DESCRIPTION OF THE INVENTION

As shown in Scheme 1, t-butyl hydrazine acetic acid (Formula 1, prepared according to Niedrich, H. et al., *Chem. Ber.* 102, 1557-1569 (1969)) was acylated with either an N-protected amino acid or dipeptide acid or any desired non-peptide carboxylic acid. The acylation reaction was carried out using a peptide coupling reagent such as an acid chloride, mixed anhydride and other methods described in The Practice of Peptide Synthesis (M. Bodanszky, Ed., 1984 Springer-Verlag, NY) to yield an acyl hydrazone (Formula 3). The acyl hydrazone in turn was reacted with an α -halo acetyl chloride (Formula 4) in the presence of a base such as N-methylmorpholine. This reaction afforded compounds of Formula 5. When compounds of Formula 5 were treated with trifluoroacetic acid (TFA) in methylene chloride (25% TFA solution) for 3 hrs. at 25 °C, the t-butyl ester was hydrolyzed and the inhibitors of Formula 7 were obtained. Alternatively, when the acyl hydrazide of Formula 5 (W=Br) was treated with a phenol, arylcarboxylic acid, diarylphosphinic acid, a tetrionic acid derivative or a 5-hydroxy-pyrazole derivative in dimethylformamide containing potassium fluoride, the compounds of the type described by Formula 6 were obtained. These t-butyl esters were then treated with TFA as before (Formula 5 \rightarrow 7) to furnish inhibitors of Formula 7.

20

SCHEME 1

5 where

W = halo;

HO-Y = phenol, carboxylic acid, diarylphosphinic acid, tetronic acid derivative or 5-hydroxypyrazole derivative;

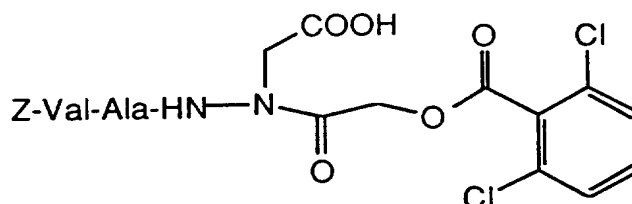
10

R¹ and R³ are as defined previously.

The following examples further illustrate the compounds of the invention.

15

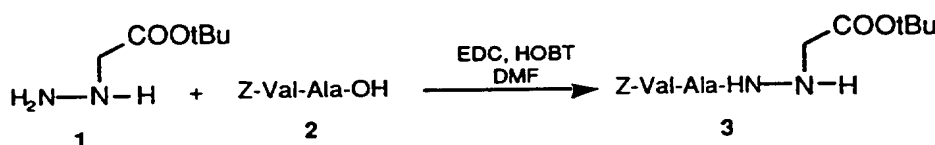
Example 1



5 Z = N terminal protecting group N-benzyloxycarbonyl

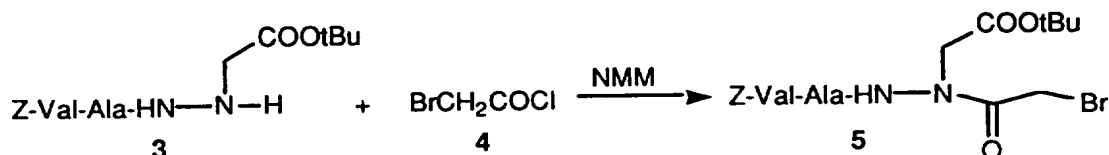
N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid 2,6-dichlorobenzoyloxymethyl ketone

10



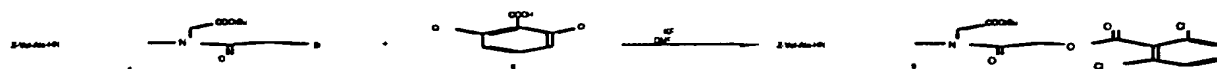
15 **Part A:** Z-Val-Ala-OH(2) (4.00 g, 12.4 mmol), hydrazine(1) (2.18 g, 14.9 mmol) and HOBT (2.09 g, 13.6 mmol) were dissolved in 25 mL of DMF. 1-(3-Dimethyl-aminopropyl)-3-ethyl carbodiimide hydrochloride (EDC, 2.61 g, 13.6 mmol) was then added in one portion at 0 °C and the reaction solution stirred for 6 h. The reaction solution was then poured into 800 mL of water and the product extracted into ethylacetate (3x). The organic layer was washed (water, 0.3 NaHSO₄ (2x), 5% NaHCO₃ (2x), brine) and dried over Na₂SO₄. Evaporation of solvent yielded⁽³⁾ as a fine white powder of sufficient purity: 5.34 g, 95%, MP 115-157 °C.

20

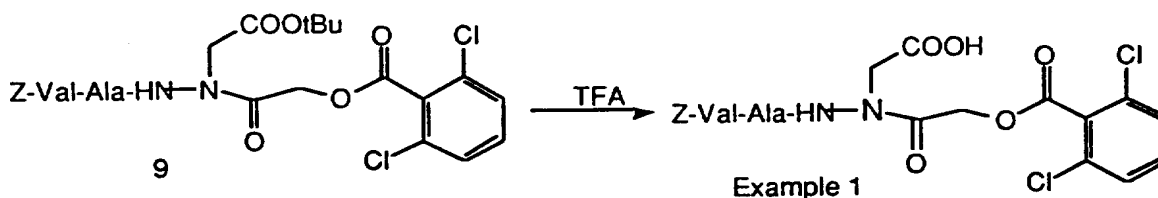


25 **Part B:** Bromoacetyl chloride(4) (0.71 mL, 8.66 mmol) was added dropwise to a 0 °C solution of hydrazide(3) (3.00 g, 6.66 mmol) and N-methylmorpholine (1.1 mL, 9.99 mmol) in dichloromethane (80 mL).

After stirring for 5 h at 0 °C, the reaction mixture was poured into water (600 mL) and the product extracted into ethylacetate (3x). The organic phase was then washed (water, 0.3 NaHSO₄ (2x), 5% NaHCO₃ (2x), brine), dried with Na₂SO₄, filtered and concentrated. Recrystallization from warm ethylacetate/hexanes provided the bromoacetyl derivative(5) (3.11 g, 82%) as a white crystalline solid, MP 136-138 °C.



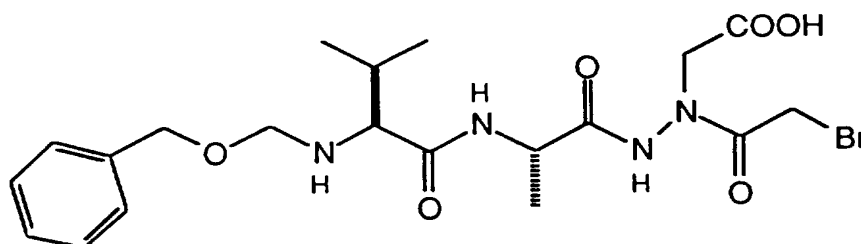
Part C: Powdered HF (0.128 g, 2.18 mmol) was added to a homogeneous solution of bromoacetyl derivative(5) (0.500 g, 0.874 mmol) and 2,6-dichlorobenzoic acid(8) (0.234 g, 1.20 mmol) in 5 mL of DMF. The reaction was then warmed at 60°C for 10 hrs. The reaction mixture was poured into 100 mL of water and the products extracted into ethylacetate (3x). The ethylacetate layer was then washed (water, 0.3 NKHSO₄ (2x), 5% NaHCO₃ (2x), brine), dried with Na₂SO₄, filtered and concentrated. Recrystallization from warm EtOAc/hexanes provided dichlorobenzoate derivative(9) as a white crystalline solid (0.510 g, 86%, MP 176-178°C.)



Part D: Toluene (5 mL) and a 25% solution of TFA/CH₂Cl₂ (100 mL) were added to a 250 mL flask containing tBu ester(9) (0.450 g, 0.66 mmol). After stirring for 1 hr at RT, additional toluene (50 mL) was added and all solvents evaporated under reduced pressure. Two additional 100 mL portions of toluene were added and evaporated. Concentration of a homogeneous solution of the product in dichloromethane and hexanes (2x) produced a fine white powder. After two-fold trituration with several mL of hexane, Example 1 was collected on a filter and dried under high vacuum: 0.411 g, 95%, amorphous. Anal. calcd. for C₂₇H₃₀Cl₂N₄O₉: C, 51.85; H, 4.83; N, 8.46. Found C, 51.66; H, 5.14; N, 8.51.

Following the procedure described in Scheme 1 and by analogy to Example 1, the following compounds were prepared.

Example 2



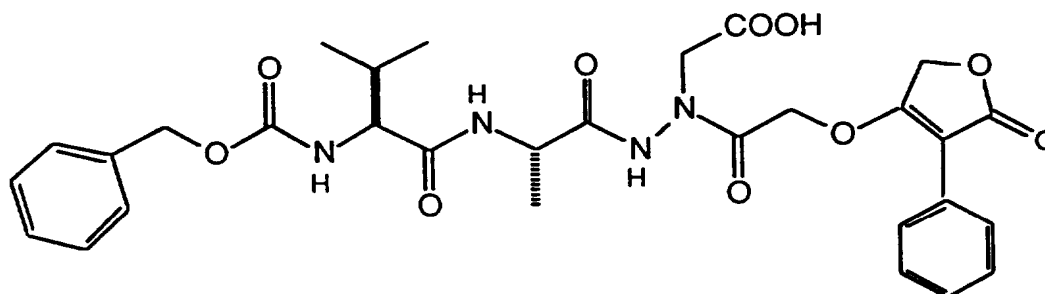
5

N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid bromomethyl ketone

10 Anal. calcd. for $C_{20}H_{27}BrN_4O_7 \cdot O_2H_2O$: C, 46.29; H, 5.32; N, 10.80. Found: C, 46.6; H, 5.50; N, 10.45.

15

Example 3



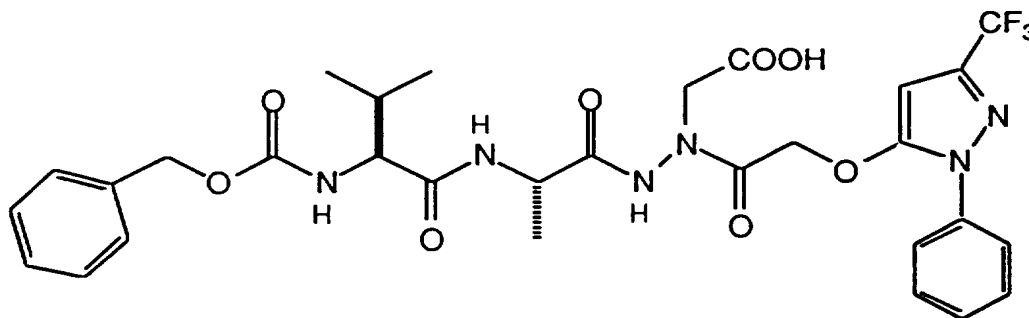
20

N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid 4-(3-phenyl)tetronyloxymethyl ketone

25

Anal. calcd. for $C_{30}H_{34}N_4O_{10} \cdot 1H_2O$: C, 57.32; H, 5.77; N, 8.91. Found: C, 57.28; H, 5.67; N, 9.02.

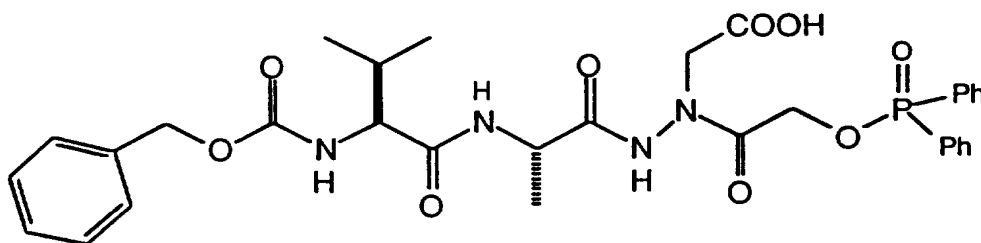
Example 4



N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid 5-(1-phenyl-3-trifluoromethyl)pyrazoloxymethyl ketone

Anal. calcd. for $C_{30}H_{33}F_3N_6O_8 \cdot 0.5 H_2O$: C, 53.65; H, 5.10; N, 12.51. Found: C, 53.94; H, 5.17; N, 12.24.

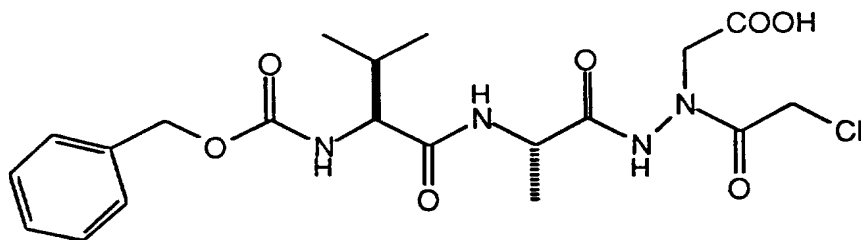
Example 5



N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid diphenylphosphinyloxymethyl ketone

Anal. calcd. for $C_{32}H_{37}N_4O_9P \cdot 0.25 H_2O$: C, 58.49; H, 5.75; N, 8.53. Found: C, 58.23; H, 5.87; N, 8.41.

Example 6

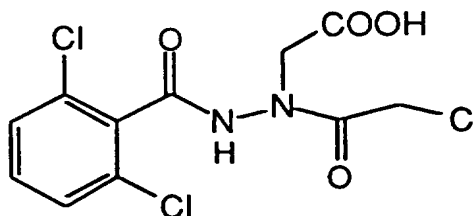


5 N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid chloromethyl ketone

mass spectrum $m/z = 471$ (M+H)

10

Example 7



15

N-(2,6-Dichlorobenzoyl)-azaaspartic acid chloromethyl ketone

20 mass spectrum $m/z = 338$ (M+H)

In Vitro Testing

25

Second order rates for inactivation were obtained by using the enzyme assay described in Dolle, R.E. et al., J. Med Chem., 37, 563 (1994).

30 The compounds in examples 1-7 possess IL-1 β protease inhibition (kobs/I were $>5,000 \text{ M}^{-1} \text{ s}^{-1}$).

In Vivo

In vivo inhibition (IC₅₀) was determined as follows:

5 Human monocytes were isolated from heparinized leukopheresis units obtained through Biological Specialty Corporation (Lansdale, PA). Monocytes were purified by Ficoll-Hopaque (Pharmacia Fine Chemicals, Piscataway, NJ) gradient centrifugation and more than 95% pure monocyte populations obtained by centrifugal elutriation. The assay was performed on duplicate samples of freshly isolated human monocytes,
10 cultured in suspension at 37 °C and rotated gently in conical bottom polypropylene tubes (Sardstedt Inc., Princeton, NJ). Human monocytes at a concentration of 5×10^6 cells/mL were resuspended in 1 mL of RPMI 1640 (a common tissue buffer from M.A. Bioproducts, Walkersville, MD) containing 1% fetal calf serum (FCS) (HyClone, Logan, UT) and 50 µg/mL gentamycin (Gibco, Grand Island, NY). The cells were treated either
15 with a compound of the invention (i.e. test compound) or with a non-inhibitor (control compound, typically 0.03% DMSO) for 15 minutes and then activated with 0.01% fixed *Staphylococcus aureus* (The Enzyme Center, Malden, MA) for 1 hour. The cells were then centrifuged and resuspended in 1 mL of cysteine, methionine-free RPMI media containing 1% dialyzed FCS (Hyclone). The cells were pretreated with a test
20 compound or control compound for 15 minutes after which 0.01% fixed *S. aureus* plus 100 µCi Tran 35-S label (ICN, Irvine, CA) was added and the cells incubated at 37 °C for 1 hour. After incubation, cells were centrifuged, washed once in phosphate buffer saline and resuspended in 1 mL RPMI containing 1% fetal calf serum. The cells were again pretreated with a test or control compound for 15 minutes and then 0.01% *S.*
25 *aureus* for 2 hours. At the end of the incubation, cells were centrifuged and supernates saved for immunoprecipitation. Cells were washed once in phosphate buffer saline and then lysed in RIPA, a continuous cell media buffer containing 2 mM phenylmethylsulfonyl fluoride, 10 mM iodoacetate, 1 µg/mL pepstatin A, 1 µg/mL leupeptin and 0.5 TIU aprotinin.

30 For the immunoprecipitations, an equal volume of 1% dry milk in RIPA buffer plus 50 µL of resuspended protein A sepharose CL-4B (Pharmacia, Piscataway, New York) was added to supernates and 1 mL of 4% dry milk containing protein A sepharose CL-4B to cell lysates and samples rotated for 30 minutes at 4 °C. Beads
35 were then centrifuged down, samples transferred to fresh tubes and incubated overnight with 40 µg rabbit anti-human IL-1β polyclonal antibody (Genzyme, Cambridge, MA). The IL-1β proteins were then precipitated with 70 µL protein A

sepharose, resuspended in 60 µL SDS sample buffer and run on 15% SGD-PAGE gels. Autoradiography was performed on dried gels and the amount of radioactivity (counts per minute, cpm) quantitated using a Betascope 603 analyzer.

5 Data Analysis

In the monocyte pulse chase assay, each test parameter was run in duplicate. Data was collected from the Beta Scope using a personal computer, then transferred to the VAX system for calculation of mean cpm and standard deviation of the mean.
10 When test compounds were evaluated, the percent inhibition of release of mature IL-1β was calculated as follows:

$$100 \times [1 - (\text{cells treated with stimuli} + \text{test compound} - \text{unstimulated cells}) / (\text{cells treated with stimuli} + \text{control compound} - \text{unstimulated cells})]$$

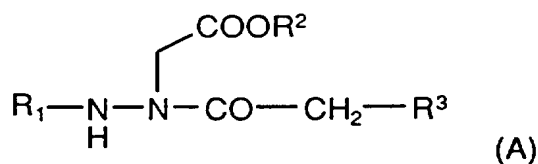
15

These % inhibition values were then used to calculate IC₅₀ value for each compound. Since the human monocyte pulse chase assay uses primary cells from different donors, each test compound was run in 2-3 separate experiments, using
20 monocytes from 2-3 different donors.

For examples given above, the *in vivo* IC₅₀'s ranged from approximately 0.1 up to approximately 10 µM.

CLAIMS:

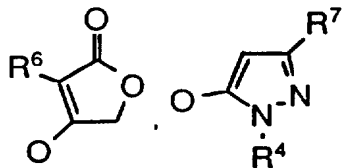
1. A compound of the formula (A) or a pharmaceutically acceptable salt thereof:



wherein:

$\text{R}_2 = \text{H}$ or alkyl;

$\text{R}^3 = \text{halo}, \text{O}(\text{CO})_{0-1} \text{ aryl}, \text{OPOR}^4\text{R}^5;$



where

R^4 and $\text{R}^5 = \text{aryl};$

$\text{R}^6 = \text{H}, \text{aryl}$ or aralkyl;

$\text{R}^7 =$ independently selected from R^6 , CF_3 and $\text{CF}_2\text{CF}_3;$

$\text{R}^1 = \text{R}^6\text{-CO}, \text{heteroaryl-CO}, \text{heteroaralkyl-CO}$ and amino acid.

2. The compound of claim 1 wherein:

$\text{R}^2 = \text{H};$

$\text{R}^3 = \text{O}(\text{CO}) \text{ aryl};$ and

$\text{R}^1 = \text{R}^6\text{-CO},$ and amino acid where R^6 is aralkyl.

3. The compound of claim 1 wherein:

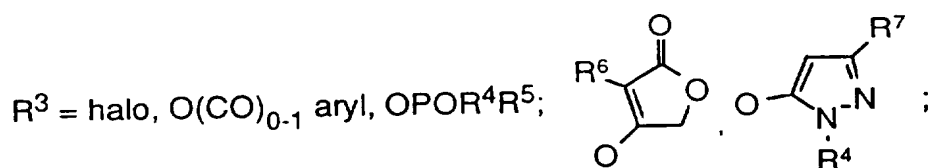
$\text{R}^2 = \text{H};$

$\text{R}^3 = \text{halo};$ and

$\text{R}^1 = \text{R}^6\text{-CO}, \text{aryl-CO}$ and amino acid where R^6 is aralkyl.

4. The compound of claim 1 wherein:

$\text{R}^2 = \text{H};$



where

$R^6 = \text{aryl};$

$R^7 = \text{CF}_3;$ and

5 $R^1 = R^6\text{-CO}$ and amino acid where R^6 is aralkyl.

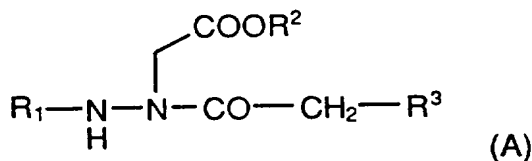
5. The compound according to claim 1 selected from the group consisting of:
 N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid 2,6-dichlorobenzoyloxymethyl ketone; N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid bromomethyl ketone;
 10 ketone; N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid 3-(2-phenyl)-tetronyloxymethyl ketone; N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid 5-(1-phenyl-3-trifluoromethyl)pyrazoloxymethyl ketone; N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid diphenylphosphinyloxymethyl ketone; N-Benzyloxycarbonyl-L-valine-L-alanine-azaaspartic acid chloromethyl ketone; and N-(2,6-Dichlorobenzoyl)-
 15 azaaspartic acid chloromethyl ketone.

6. A pharmaceutical composition for inhibiting interleukin- 1β protease comprising a compound of the formula (A) defined in claim 1 in a pharmaceutically acceptable carrier.

20

7. A pharmaceutical composition for inhibiting interleukin- 1β protease comprising the compound defined in any of claims 2-5.

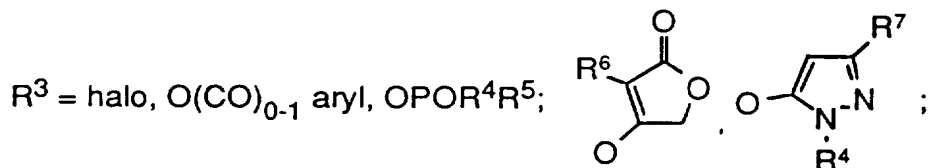
8. A method for inhibiting interleukin- 1β protease activity in a mammal in need of
 25 treatment for inflammatory and immune-based diseases of lung, central nervous system, and connective tissue comprising administering to said mammal an effective interleukin- 1β inhibitory amount of a composition comprising a compound of the formula (A) or a pharmaceutically acceptable salt thereof.



30

wherein:

$R_2 = \text{H or alkyl};$



where

$R^4 \text{ and } R^5 = \text{aryl};$

$R^6 = \text{H, aryl or aralkyl};$

$R^7 = \text{independently selected from } R^6, \text{CF}_3 \text{ and CF}_2\text{CF}_3;$

$R^1 = R^6\text{-CO, heteroaryl-CO, heteroaralkyl-CO and amino acid.}$

9. A method for inhibiting interleukin-1 β protease activity in a mammal in need of treatment for inflammatory and immune-based diseases of lung, central nervous system, and connective tissue comprising administering to said mammal an effective interleukin-1 β inhibitory amount of a composition comprising a compound defined in any of claims 2-5.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09668**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/18, 19, 20, 119, 407, 471, 542, 551, 563, 565; 530/323, 331; 548/370.1; 549/318; 560/37, 169; 562/442, 561

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CHEMICAL ABSTRACTS, DIALOG

search terms: formula (A), azaaspartic, interleukin, protease, convertase, inhibit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,430,128 (CHAPMAN ET AL) 04 July 1995, see entire document.	1-9
A,P	US, A, 5,434,248 (CHAPMAN ET AL) 18 July 1995, see entire document.	1-9
A	Bioorganic & Medicinal Chemistry Letters, Volume 2, Number 6, issued 1992, Chapman, "Synthesis Of A Potent, Reversible Inhibitor Of Interleukin-1 β Converting Enzyme", pages 613-618.	1-9
A	Chemische Berichte, Volume 102, issued 1969, Niedrich, "Synthese von substituierten 2.4-Bis-carboxymethyl-1-acyl-semicarbaziden, den α -Aza-asparagin-Peptiden", pages 1557-1569.	1-9

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

03 OCTOBER 1995

Date of mailing of the international search report

24 NOV 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

JEFFREY E. RUSSEL

Facsimile No. (703) 305-3230

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09668

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Tetrahedron Letters, Volume 26, Number 26, issued 1985, Moody et al, "Synthesis Of Aza- β -Lactams By Rhodium Carbenoid Mediated Cyclisation", pages 3171-3172.	1-9
A	Chemical Abstracts, Volume 88, Number 5, issued 30 January 1978, Dutta et al, " α -Aza-analogs of biologically-active peptides", abstract no. 38140a, Pept., Proc. Eur. Pept. Symp., 14th, 1976, pages 517-522.	1-9
A	Chemical Abstracts, Volume 78, Number 11, issued 19 March 1973, Niedrich et al, "Hydrazine compounds as hetero-components in peptides. XIII. N ^α -Acylation of hydrazinoacetic acid derivatives and synthesis of eledoisin octapeptides with N ^α -acetylated hydrazinoacetic acid instead of asparagine and glycine", page 515, column 2, abstract no. 72578c, J. Prakt. Chem., 314(5-6), pages 735-50.	1-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09668

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 31/15, 31/195, 31/22, 31/235, 31/34, 31/415, 31/66, 38/05, 38/06; C07K 5/02, 5/062, 5/083; C07C 243/26, 243/36, 243/38; C07D 231/04, 231/08, 231/20, 307/60

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/18, 19, 20, 119, 407, 471, 542, 551, 563, 565; 530/323, 331; 548/370.1; 549/318; 560/37, 169; 562/442, 561